

On-orbit Quantitative Real-time Gene Expression Analysis using the WetLab-2 System

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NASA Ames Research Center's WetLab-2 Project enables on-orbit quantitative Reverse Transcriptase PCR (qRT-PCR) analysis without the need for sample return. The WetLab-2 system is capable of processing sample types ranging from microbial cultures to animal tissues dissected on-orbit. The project developed a RNA preparation module that can lyse cells and extract RNA of sufficient quality and quantity for use as templates in qRT-PCR reactions. Our protocol has the advantage of using non-toxic chemicals and does not require alcohols or other organics. The resulting RNA is dispensed into reaction tubes that contain all lyophilized reagents needed to perform qRT-PCR reactions. System operations require simple and limited crew actions including syringe pushes, valve turns and pipette dispenses. The project selected the Cepheid SmartCycler®, a Commercial-Off-The-Shelf (COTS) qRT-PCR unit, because of its advantages including rugged modular design, low power consumption, rapid thermal ramp times and four-color multiplex detection. Single tube multiplex assays can be used to normalize for RNA concentration and integrity, and to study multiple genes of interest in each module. The WetLab-2 system can downlink data from the ISS to the ground after a completed run and uplink new thermal cycling programs.

The ability to conduct qRT-PCR and generate results on-orbit is an important step towards utilizing the ISS as a National Laboratory facility. Specifically, the ability to get on-orbit data will provide investigators with the opportunity to adjust experimental parameters in real time without the need for sample return and re-flight. On orbit gene expression analysis can also eliminate the confounding effects on gene expression of reentry stresses and shock acting on live cells and organisms or the concern of RNA degradation of fixed samples and provide on-orbit gene expression benchmarking prior to sample return. Finally, the system can also be used for analysis of air, surface, water, and clinical samples to monitor environmental pathogens and crew health. The validation flight of the WetLab-2 system using *E. coli* bacteria and mouse liver launched on SpaceX-7 in June 2015 and will remain on the ISS National Laboratory.

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